TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.:	PCT/BE96/00087
International Filing Date:	August 14, 1996
Priority Date Claimed:	August 15, 1995
Title of Invention:	NUCLEIC ACID MOLECULES ENCODING PEPTIDES HAVING PRONOCICEPTIVE PROPERTIES
Applicant(s) for DO/EO/US:	Marc Parmentier, Gilbert Vassart, Jean-Claude Meunier and Catherine Mollereau

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.

2. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

3. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

4. (X) A copy of the International Application as filed (35 USC 371(c)(2))
   a. () is transmitted herewith (required only if not transmitted by the International Bureau).
   b. (X) has been transmitted by the International Bureau.
   c. () is not required, as the application was filed in the United States Receiving Office (RO/US).

5. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))
   a. () are transmitted herewith (required only if not transmitted by the International Bureau).
   b. () have been transmitted by the International Bureau.
   c. () have not been made; however, the time limit for making such amendments has NOT expired.
   d. (X) have not been made and will not be made.

6. (X) Eleven (11) pages drawing.

7. (X) A copy of the International Preliminary Examination Report with any annexes thereto, such as any amendments made under PCT Article 34.

Items 8. to 14. below concern other document(s) or information included:

8. (X) A FIRST preliminary amendment.

9. (X) Diskette (computer readable form) of the Sequence Listing.

   Please Note: Pursuant to 37 C.F.R § 1.821(f), the undersigned hereby verifies that the information in the computer readable form is the same as the information included in the paper copy of the Sequence Listing included within the enclosed preliminary amendment.

10. (X) International Application as published.

11. (X) PCT Form PCT/IPEA/402, 401, 409 and 416.

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12. (X) PCT Form PCT/IB/308, 301 and 332.

13. (X) PCT request form.

14. (X) PCT Form PCT/ISA/202, 210 and 220.

15. (X) A return prepaid postcard.

16. (X) The following fees are submitted:

17. (X) PCT FORM PCT/IB/304

				FEES
	BASIC FEE			$930
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**	
Total Claims	24 - 20 =	4 ×	$22	$88
Independent Claims	3 - 3 =	0 ×	$82	$0
	TOTAL OF ABOVE CALCULATIONS		$1,018	
	TOTAL FEES ENCLOSED			$930

17. (X) The fee for later submission of the signed oath or declaration set forth in 37 CFR 1.492(e) will be paid upon submission of the declaration.

18. (X) A check in the amount of $930.00 to cover the above fees is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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VANMA72.001APC PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Parmentier, et al.	Group Art Unit Unknown
Int'l App. No.	:	PCT/BE96/00087	
Int'l Filing Dte.	:	August 14, 1996	
For	:	NUCLEIC ACID MOLECULES ENCODING PEPTIDES HAVING PRONOCICEPTIVE PROPERTIES	
Examiner	:	Unknown	

PRELIMINARY AMENDMENT

Hon. Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-captioned application, please amend the application as follows:

IN TEH SPECIFICATION:

Page 1, between the title of the invention and the first sentence of the description, please insert --This is a §371 application of PCT/BE96/00087.--.

IN THE SEQUENCE LISTING:

Between page 27 and 28 of the present specification, please insert the attached Sequence Listing having page numbers 27-29. Please renumber pages 27 to 33 of the present specificaiton as pages 30 to 36.

IN THE CLAIMS:

Please cancel Claims 1-34.

Please add the following claims:

35. An isolated polynucleotide which corresponds to at least 70% of the SEQ ID NO:1 or its complementary strand.

36. An isolated polynucleotide according to Claim 35, which corresponds to at least 90% of the SEQ ID NO:1 or its complementary strand.

37. An isolated polynucleotide comprising at least the SEQ ID NO:1, its complementary strand, or a portion thereof having more than 15 nucleotides able to identify or reconstitute SEQ ID NO:1 or its complementary strand.

38. An isolated peptide encoded by an isolated polynucleotide according to Claim 35.

39. An isolated peptide according to Claim 38, comprising the peptide listed as SEQ ID NO:2 or agonists of a receptor or receptors of said peptide.

40. An isolated peptide according to Claim 39, which is a ligand of the ORL_1 receptor.

41. An isolated peptide according to Claim 38, comprising the peptide listed as SEQ ID NO:3 or agonists of a receptor or receptors of said peptide.

42. An isolated peptide according to Claim 38, comprising the peptide listed as SEQ ID NO:4 or agonists of a receptor or receptors of said peptide.

43. An inhibitor directed against an isolated polynucleotide according to Claim 35, an isolated peptide encoded by said isolated polynucleotide, or a receptor or receptors of said isolated peptide.

44. An inhibitor according to Claim 43, which is a polyclonal or monoclonal antibody, or a portion thereof, directed against said isolated peptide or a receptor or receptors of said isolated peptide.

45. An inhibitor according to Claim 43, which is an antigens oligonucleotide which has a sequence capable of specifically binding to said isolated polynucleotide to prevent its transcription and/or its translation.

46. An inhibitor according to Claim 43, which is an antagonist to a receptor of said isolated peptide.

47. A vector comprising an isolated polynucleotide according to Claim 35.

48. A pharmaceutical composition comprising an element selected from the group consisting of an isolated polynucleotide according to Claim 35, an isolated peptide encoded by said isolated polynucleotide, an inhibitor directed against said isolated polynucleotide,

and a vector comprising said isolated polynucleotide, and a pharmaceutically acceptable carrier.

49. A pharmaceutical composition according to Claim 48, for the treatment and/or the prevention of a disease related to at least one function or behavior selected from the group consisiting of hyperalgesia, neuroendocrine secretion, stress, locomotor activity, anxiety, instinctive behavior, decreasing of learning, memory, curiosity, attention, and sensory perception.

50. A transgenic non-human animal, comprising an isolated polynucleotide according to Claim 35.

51. A method for recovering an antagonist or an agonist of an isolated peptide according to the Claim 38, said antagonist or said agonist being capable of specifically binding to a receptor present on a cell surface, said method comprising the steps of:

preparing a cell extract from cells comprising a vector adapted for expression in said cells, said vector comprising a polynucleotide which expresses said receptor on the cells' surface;

isolating a membrane fraction from said cell extract;

incubating compounds present within said membrane fraction under conditions permitting a peptide known to bind specifically to said receptor;

detecting the presence of compounds, if any, bound to said receptor; and

recovering said bound compounds as the antagonist or the agonist.

52. A method for recovering an antagonist or an agonist of an isolated peptide according to the Claim 38, said antagonist or said agonist being capable of specifically binding to an receptor present on a surface of cells to prevent said isolated peptide from activating said receptor, said method comprising the steps of:

contacting a cell comprising a vector adapted for expression in said cell, with a compound under conditions permitting measuring a functional response, said vector comprising a polynucleotide which expresses said receptor on the cell's surface;

determining whether the compound prevents said isolated peptide to activate said receptor; and

recovering the compound as the antagonist or the agonist if said compound does not activate said receptor.

53. An antagonist or an agonist of an isolated peptide according to the Claim 38, said antagonist or said agonist being capable of specifically binding to a receptor present on a cell surface, said antagonist or said agonist being obtained by a method comprising the steps of:

preparing a cell extract from cells comprising a vector adapted for expression in said cells, said vector comprising a polynucleotide which expresses said receptor on the cells' surface;

isolating a membrane fraction from said cell extract;

incubating compounds present within said membrane fraction under conditions permitting a peptide known to bind specifically to said receptor;

detecting the presence of compounds, if any, bound to said receptor; and

recovering said bound compounds as the antagonist or the agonist.

54. An antagonist or an agonist of an isolated peptide according to the Claim 38, said antagonist or said agonist being capable of specifically binding to an receptor present on a surface of cells to prevent said isolated peptide from activating said receptor, said antagonist or said agonist being obtained by a method comprising the steps of:

contacting a cell comprising a vector adapted for expression in said cell, with a compound under conditions permitting measuring a functional response, said vector comprising a polynucleotide which expresses said receptor on the cell's surface;

determining whether the compound prevents said isolated peptide to activate said receptor; and

recovering the compound as the antagonist or the agonist if said compound does not activate said receptor.

55. A pharmaceutical composition comprising an antagonist or an agonist according to Claim 53, and a pharmaceutically acceptable carrier.

56. A pharmaceutical composition comprising an antagonist or an agonist according to Claim 54, and a pharmaceutically acceptable carrier.

57. A diagnostic and/or dosage device, comprising:

an inhibitor which is directed against an isolated polynucleotide according to Claim 35, an isolated peptide encoded by said isolated polynucleotide, or a receptor or receptors of said isolated peptide;

an isolated peptide encoded by said isolated polynucleotide; and

optionally a receptor or receptors of said isolated peptide.

58. A method of genetic treatment or prevention of a disease induced by an isolated polynucleotide according to Claim 35 or an isolated peptide encoded by said isolated polynucleotide in an animal, said method comprising the step of administering to a patient having said disease an inhibitor or a polynucleotide encoding said inhibitor with a pharmaceutically acceptable carrier to reduce the expression and/or effects resulting from expression of said polynucleotide encoding or said peptide, said inhibitor being directed against said isolated polynucleotide, an isolated peptide, or a receptor or receptors of said isolated peptide.

REMARKS

Claims 1-34 have been canceled without prejudice, and replaced with new Claims 35-58. The amendments requested herein are requested in order to adapt the claims to U.S. practice. As such, these amendments do not constitute the addition of new matter to the application, and entry of the amendments is respectfully requested. In addition, the

Sequence Listing has been incorporated into the specificaiton, and entry of the Sequence Listing is respectfully requested.

Verification Under 37 C.F.R. §1.821(f)

Pursuant to 37 C.F.R. §1.821(c) and (e), the Sequence Listing has been submitted in paper and in a computer readable form. Pursuant to 37 C.F.R.§1.821(f), the undersigned hereby verify that the content of the paper and computer readable copies are the same.

Should the Examiner have any questions concerning the foregoing, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: February 13, 1998

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NUCLEIC ACID MOLECULES ENCODING PEPTIDES HAVING PRONOCICEPTIVE PROPERTIES

This application claims the benefit of U.S. Provisional Patent Application No. 60/002,368 filed on August 15, 1995.

Field of the invention.

The present invention is related to nucleic acid molecules, the peptides encoded by said nucleic acid molecules and the inhibitors directed against said nucleic acid molecules or said peptides.

The present invention concerns also the pharmaceutical composition, the diagnostic and/or dosage device comprising said products and methods for identifying the inhibitors according to the invention.

Various opioid receptors were described and used for the screening of improved drugs.

The present invention is related to a new nucleic acid, especially a nucleic acid encoding peptides which have less than 70% homology with the peptides with are known to be ligands of the opioid receptors, said peptides having pronociceptive properties.

The inhibitors of said nucleic acid molecules or

encoded peptides could be used as new types of drugs in the control of various behaviours or functions such as neuroendocrine secretion, stress, learning and memory, attention and emotions, homeostasis, sensory perception, motricity (locomotion), anxiety, instinctive behaviours, hyperalgesia or hypoalgesia, ...

Summary of the invention

The present invention is related to a nucleic acid molecule which corresponds to at least 70%, preferably at least 90%, of the SEQ ID NO. 1 or its complementary strand.

Preferably, the nucleic acid molecule according to the invention is an isolated nucleic acid molecule comprising at least the sequence SEQ ID NO 1. or its complementary strand or a portion thereof.

It is meant by a "nucleic acid molecule", a RNA or a DNA such as a cDNA or a genomic DNA.

It is meant by a "portion of an isolated nucleic acid molecule", any kind of nucleic acid molecule which is specific of SEQ ID NO. 1 such as a probe or one or several primer(s) which could be used in order to identify and reconstitute said specific isolated nucleic acid molecule, for instance by genetic amplification (PCR, LCR, CPR, ...) or by specific probe hybridization. Therefore, a portion of said nucleic acid molecule is any portion of said nucleic acid molecule having more than 15 nucleotides and which is specific of SEQ ID NO. 1.

The present invention concerns also any peptide encoded by the nucleic acid molecule according to the invention. Therefore, said peptide may be a precursor of an active peptide which means a peptide which should be cleaved at a specific side in order to be active. An example of said

peptide is the prepronociceptine described hereafter which is cleaved into two active heptadecapeptides according to the invention.

A preferred embodiment of the present invention concerns the peptide which has the amino sequence of SEQ ID NO. 2 : Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln or agonists thereof, said peptide being a ligand of the ORL_1 receptor, preferably a ligand of the mammal ORL_1 receptor, more specifically a ligand of the human ORL_1 receptor.

Another embodiment of the present invention concerns the peptide which has the amino sequence of SEQ ID NO. 3 : Phe-Ser-Glu-Phe-Met-Arg-Gln-Tyr-Leu-Val-Leu-Ser-Met-Gln-Ser-Ser-Gln, or agonists.

A further embodiment of the present invention concerns a peptide which has the amino sequence of SEQ ID NO. 4 : Thr-Leu-His-Gln-Asn-Gly-Asn-Val, or agonists.

An agonist according to the invention, may be a molecule which mimics the peptide interaction with its receptor(s). Such may be analogs or fragments of the peptide according to the invention, or antibodies directed against the ligands binding side epitopes of the peptide receptors, or anti-idiotypic antibodies directed against particular antibodies which bind to receptor-interacting specific portions of the peptide according to the invention.

Said antibodies can be raised to the peptide fragments and analogs, both in their naturally occurring forms, and in their recombinant forms. Additionally, antibodies can be raised to the peptide either in its active forms or in its inactive forms.

Another aspect of the present invention concerns

the inhibitor directed against the nucleic acid molecule or the peptide according to the invention. An inhibitor is any kind of molecule which is able to avoid translation of the nucleic acid molecule according to the invention or avoid or reduce the interaction of the peptide or a portion of said peptide to its receptor.

Advantageously, the inhibitor according to the invention ~~is~~ a polyclonal or a monoclonal antibody or a portion thereof, especially the epitope portion of an antibody such as the portions Fab', Fab, $F(ab')_2$, of said antibody.

According to another embodiment of the present invention, said inhibitor is any molecule which may interact with the nucleic acid molecule according to the invention and prevent its expression in a cell of an animal, such as a mammalian, preferably a human.

Advantageously, said inhibitor is an antisense oligonucleotide which has a sequence capable of specifically binding to the nucleic acid molecule according to the invention so as to prevent its expression (its transcription and/or its translation).

Preferably, said inhibitor comprises chemical analogs of nucleotides having advantageously sequences which differ from one another at predefined positions. Said oligonucleotides could be coupled to a substance such as a rybozyme which inactivates the nucleic acid according to the invention.

Said inhibitor could also be an antagonist to the receptor of the peptide according to the invention. An antagonist is a ligand of said receptor which blocks the interaction of the peptide to its receptor.

The present invention is also related to a vector comprising the nucleic acid molecule according to the invention.

Another aspect of the present invention is related to a pharmaceutical composition comprising an element chosen among the group consisting of the nucleic acid molecule, the peptide, the inhibitor or the vector according to the invention and a pharmaceutically acceptable carrier.

A pharmaceutical carrier can be any compatible non-toxic substance suitable for delivering the composition of the invention to a patient.

Preferably, said pharmaceutical composition comprises also an amount of a substance effective to reduce the expression and/or the "effects" resulting from the expression of the peptide or the nucleic acid molecule according to the invention.

The dosage of the active ingredients in the pharmaceutical composition may vary according to the pharmaceutically acceptable carrier used, the patient treated and the side effects of said active ingredients.

The pharmaceutical composition according to the invention is advantageously used for the treatment and/or the prevention of a disease related to the following functions and/or behaviours : hyperalgesia, hypoalgesia, neuroendocrine secretion, stress, anxiety, instinctive behaviours, decrease of learning, memory, locomotor activity, curiosity, attention and/or sensory perception.

The present invention is also related to a transgenic non human animal which comprises the nucleic acid molecule according to the invention or the vector according to the invention. Said vector adapted for expression in a cell or in a transgenic non human animal is any molecule or

microorganism which may transfect said cell or said animal and may express in said cell or said animal the exogenous nucleic acid molecules it comprises. Said vector may be a plasmid, a recombinant virus, a baculovirus, an adenovirus, ...

Another aspect of the present invention of the present invention is related to a method for recovering an inhibitor not known to be capable of specifically binding to the peptide according to the invention can specifically bind to it; said method comprises contacting the peptide according to the invention under conditions permitting binding of an inhibitor known to bind the peptide according to the invention, determining the presence of any inhibitor bound to said peptide and recovering said inhibitor.

The present invention is also related to a method for recovering a compound not known to be capable of specifically binding as an antagonist or as an agonist of the peptide according to the invention, specifically the peptide of an ORL_1 receptor, preferably a mammal ORL_1 receptor, specifically a human ORL_1 receptor, can specifically bind to said receptor; said method comprises contacting a cell, preferably a mammalian cell, comprising the vector adapted for expression in a mammalian cell, which vector further comprises nucleic acid molecules which express said ORL_1 receptor on the cell surface with the compound under conditions permitting biding of the peptide known to bond to said receptor, and detecting the presence of any compound bound to said receptor and recovering said compound.

According to another embodiment of the present invention, said method comprises preparing a cell extract from a cell, preferably a mammalian cell, which comprises a vector adapted for expression in said cell, said vector

further comprises nucleic acid molecules which express the receptor of the peptide according to the invention on the cell surface, isolating a membrane fraction from the cell extract, incubating the compound with the membrane fraction under conditions permitting the binding of the peptide known to bind to said receptor and detecting the presence of any bound compound and recovering said compound.

The present invention concerns also a method for recovering a compound not known to be capable of binding as an antagonist or as an agonist of the peptide according to the invention to a ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically a human ORL_1 receptor, and prevent the peptide according to the invention to activate said receptor, which comprises contacting a cell, preferably a mammalian cell, which cell comprises a vector adapted for expression of said cell, such vector further comprising nucleic acid molecules which express said receptor on the cell surface with the compound under conditions permitting measure of a functional response, determining whether the compound prevents the peptide to activate said receptor, and recovering said compound.

According to said method, the cell is a non neuronal cell comprising the cellular components necessary to produce a second messenger and wherein the determination (of whether the compound blocks the activation of the ORL_1 receptor by a peptide according to the invention or mimics inactivation of the ORL_1 receptor by a peptide according to the invention) comprises means for detecting the changes in the concentration of the second messenger, which is preferably chosen among the group consisting of cyclic AMP, inositol phosphate metabolite or intracellular calcium. the

modification of said second messenger is preferably monitored by a secondary production of a report molecule (such as a luciferase, a β-galactosidase, a chloramphenicol acetyltransferase, a grove hormone, ...) or by the physiological modification of the cell preferably monitored by measure of the extra-cellular pH.

Preferably, the non neuronal cell used in the method according to the invention is CHO.

The present invention concerns also the compound identified by the method according to the invention and the pharmaceutical composition comprising said compound and a pharmaceutically acceptable carrier.

The present invention concerns also the diagnostic and/or dosage device which comprises the inhibitor, the peptide and possibly their receptor(s), preferably the ORL_1 receptor according to the invention.

A last aspect of the present invention is a method of genetic treatment or prevention of a disease induced by the peptide or the nucleic acid sequence according to the invention in an animal, specifically in a human, wherein an inhibitor or a nucleic acid molecule encoding said inhibitor is administered to a patient with a pharmaceutically acceptable carrier to reduce the expression and/or the "effects" resulting from expression of said peptide or said nucleic acid sequence.

Short description of the drawings

The figure 1 represents the effects of repeated i.c.v. injections of mouse antisense mAS or of human missense hAS oligodeoxynucleotide on the latencies to rearing and escape jumping of mice in the hot plate test of Eddy and

Leimbach [11].

The figure 2 represents the inhibition by etorphin of forskolin (FSK)-induced accumulation of cAMP in CHO(ORL_1^+), a recombinant CHO cell line stably expressing orphan receptor ORL_1.

The figure 3 represents the purification of endogenous ligand of the orphan receptor ORL_1.

The figure 4 represents the inhibition by the synthetic heptadecapeptide (s-HpDPep) and its ^{1}Tyr analog of forskolin-induced accumulation of cAMP in recombinant CHO(ORL_1^+) cells.

The figure 5 represents the effects of a single i.c.v. injection of the synthetic heptadecapeptide (s-HpDPep) on the latencies to rearing and escape jumping of mice in the hole plate assay [11].

The figure 6 represents the comparison of the sequences of the endogenous heptadecapeptide referred to here as ORL_1-HpDPep, and of dynorphin. The sequence of the dynorphin is that of porcine pituitary dynorphin A^9.

The figure 7 represents the general organisation of prepronociceptin (PPNOC gene) and a comparison between the translated regions of the human prepronociceptin (hPPNOC)-enkephalin (hPPENK), dynorphin (hPPDYN) and opioimelanocortin (hPPOMC) genes.

The figure 8 represents the tissue specific expression of rat prepronociceptin mRNA by Northern blot analyses.

The figure 9 represents the human chromosome 8 and the

physical map of its short arm in the neighbourhood of the prepronociceptin gene (PPNOC).

The figure 10 represents the effects of increasing doses of nociceptin on horizontal locomotor activity in mice.

The figure 11 represents the effects of naloxone on nociceptin-induced stimulation of horizontal locomotor activity in mice.

The figure 12 represents the effects of increasing doses of SCH 23390 on nociceptin-induced stimulation of horizontal locomotor activity in mice.

The figure 13 represents the effects of increasing doses of haloperidol on nociceptin-induced stimulation of horizontal locomotor activity in mice.

Description of the invention.

1. Ligand of the ORL_1 receptor

The ORL1 receptor is an orphan receptor whose human [1] and murine [2-8] cDNAs have been recently characterized. ORL_1 (Opioid Receptor-Like 1) is structurally akin to opioid receptors and has been shown to be negatively coupled with adenylate cyclase [1]. ORL_1 transcripts are abundant in a number of brain regions [6, 18, 20] such as the central nervous system, especially in limbic areas, hypothalamus, pons and spinal cord, suggesting that the ORL_1 receptor may regulate a number of central processes including learning and memory, attention and emotions, homeostasis and sensory perception.

The Inventors describe hereafter the isolation of

a peptide according to the invention, which is a ligand of the ORL_1 receptor. Said naturally occurring ligand was purified on the basis of its anticipated ability to inhibit forskolin-induced cAMP accumulation in stable recombinant CHO(ORL_1^+) but not in non recombinant CHO(ORL_1^-) cells. The ligand is a novel neuropeptide which resembles the endorphin dynorphin A [9, 19] and whose amino acid sequence is F-G-G-F-T-G-A-R-K-S-A-R-K-L-A-N-Q. Two other peptides were also isolated. Their amino acid sequences are : F-S-E-F-M-R-Q-Y-L-V-L-S-M-Q-S-S-Q and T-L-H-Q-N-G-N-V. The first synthetic heptadecapeptide inhibits adenylate cyclase with an $IC_{50} \leq 1$ nM in CHO(ORL^+) cells in culture and, when administered in vivo, induces hyperalgesia in mice. The latter effect is consistent with the observation that in vivo inhibition of ORL_1 expression with an antisense oligonucleotide induces hypoalgesia in these animals. Taken together, our data support the notion that the first discovered heptadecapeptide is a potent ORL_1 receptor agonist and that it is endowed with pronociceptive properties. The second discovered heptadecapeptide presents also pronociceptive properties.

Comparison of the primary structures of ORL_1 and of mu-, delta- and kappa-opioid receptors revealed numerous amino acid identities, not only in the putative transmembrane domains but also in the four putative cytoplasmic loops. Although ORL_1 does not resemble more one subtype of opioid receptor than the two others, it displays many acidic amino acid residues in its second exofacial loop, a trait that singles the kappa out of opioid receptor types. The orphan receptor, ORL_1, mediates inhibition of forskolin-induced accumulation of cAMP by the opiate etorphine in a recombinant

CHO cell line stably expressing the receptor [1]. However, etorphine is 2 to 3 orders of magnitude less potent in inhibiting the cyclase via the ORL_1 than via an opioid receptor.

The Inventors have obtained evidence for a possible involvement of ORL_1 in the perception of pain. The strategy used was that of the antisense oligonucleotide [10] to inhibit expression of the ORL_1 receptor. Repeated in vivo treatment with an antisense oligonucleotide to ORL_1 mRNA rendered mice less reactive to thermal nociceptive stimulation. Figure 1 shows that, in the hot plate assay of Eddy and Leimbach [11], the animals which had been treated with antisense oligonucleotide mAS[25,9] displayed substantially increased latencies to rearing and escape jumping in comparison with saline-treated animals : 38 ± 2 ($p < 0.001$) vs 20 ± 2 sec and 108 ± 6 ($p < 0.001$) vs 71 ± 6 sec, respectively. Most significantly, the "missense" oligonucleotide hAS[25,9], the human counterpart of mAS[25,9], was totally ineffective in this respect, indicating that the hypoalgesic effects elicited by the antisense mAS[25,9] were not due to non specific actions. Since a treatment with the antisense oligonucleotide should have decreased expression of the receptor, it could be predicted that ORL_1 normally facilitates pain perception.

Owing to the potential importance of this notion in neurophysiology and, possibly, neurophysiopathology, identification of an endogenous ligand of the ORL_1 receptor had become a major issue.

The strategy for isolating an endogenous ligand of ORL1 was based on the fact that the orphan receptor is negatively coupled with adenylate cyclase. The desired compound was therefore expected to inhibit forskolin-induced

accumulation of cAMP in the recombinant CHO(ORL_1^+) but not in the non recombinant CHO(ORL_1^-) cell line, as previously shown for etorphine (Fig. 2). The choice of the initial extraction procedure from rat brain was largely based on the structural homology of ORL_1 with opioid receptors in general and the kappa receptor in particular. Extracellular loop 2 of the kappa-opioid receptor is required for high affinity binding of dynorphins [12, 13]. Since ORL_1 possesses such an acidic second exofacial loop, the ligand in question might be a peptide which resembled dynorphin. Therefore, we used the extraction procedure that allowed Teschemacher et al. to isolate a pituitary peptide that was later identified as dynorphin A [9].

The first purification step, size exclusion chromatography of the crude peptide extract on Bio-Gel P-2, proved efficient in revealing the desired activity, i. e. inhibition of cAMP accumulation in CHO(ORL_1^+) but not in CHO(ORL_1^-) cells (Fig. 3a). The active fractions were recovered in the void volume (pool F1) and, to some extent in pool F2 (not shown), indicating that the biologically active substance had a M_r around 1,800, the nominal exclusion limit of Bio-Gel P-2. Pools F3 to F10 were either inactive or equally effective in inhibiting (or stimulating in the case of F7) adenylate cyclase in the two CHO cell lines (data not shown). Pool F1 was further purified by cation exchange FPLC (Fig. 3b). Activity was recovered in two consecutive 1-ml fractions eluted at nearly 0.4 M NaCl, indicating the strongly basic nature of the active compound(s). These two fractions were then applied directly onto a reversed phase HPLC column and gradient-eluted with acetonitrile (Fig. 3c) to yield enough material of sufficient purity for protein sequencing. This material was found to be a heptadecapeptide

of average molecular mass 1,810 and whose sequence was determined to be Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln.

To make sure that the sequence in question was not that of a major contaminant, the heptadecapeptide was synthetized as well as its ^{1}Tyr analogue for the purpose of generating a radioiodinated probe. The two peptides were obtained at ≥ 98% purity as assessed by RP-HPLC and had the predicted molecular masses (1,809 and 1,825 respectively) as assessed by mass spectrometry. Fig. 4 shows that the synthetic heptadecapeptide was very potent in inhibiting forskolin-induced accumulation of cAMP in the recombinant CHO(ORL_1^+) cell line. Its IC_{50} was 0.9 x 10^{-9} mol/l and maximal inhibition amounted to 90%. The synthetic peptide had no action on cyclase in the non recombinant CHO(ORL_1^-) cell line at concentration up to 1 μM. Interestingly, the ^{1}Tyr analogue was as effective as the parent peptide on cyclase in CHO cells expressing the receptor (IC_{50} = 1.0 x 10^{-9} mol/l, maximal inhibition > 90%) and totally inactive in the wild type cells.

The synthetic heptadecapeptide was also found to be active in vivo. Intracerebroventricular injection of 10 or 100 ng of the peptide rendered mice hyperreactive in the hot plate test [11]. Figure 5 shows that the hyperalgesic effect was dose dependent for 10 ng (5.5 pmol) and 100 ng (55 pmol) of peptide. A highly significant reduction of the latencies to rearing and escape jumping was observed at the larger dose of peptide: 14 ± 2 (-36%, $p < 0.01$) vs 22 ± 2 and 48 ± 2 (-26%, $p < 0.001$) vs 65 ± 3 sec, respectively. These effects were exactly the opposite of those produced by in vivo inhibition of expression of the ORL_1 receptor (Fig. 1).

The heptadecapeptide sequence did not exist in data

banks although it was found to bear some resemblance with those of dynorphins, especially dynorphin A (Fig. 6). The structural homologies between this novel peptide and dynorphin A support the idea that the former may interact with the ORL_1 receptor as the latter does with the kappa-opioid receptor [12, 13, 15]. In particular, the novel peptide may be viewed as made up of a N-terminal Phe-Gly-Gly-Phe "message" for biological activity, followed by a Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys "address", for enhanced potency [15]. The "address" contains all the basic amino acid residues that are anticipated to bind the acidic second exofacial loop of the ORL_1 receptor.

The sequence of the endogenous ligand of ORL1 is likely to be highly conserved across species. Indeed, the Inventors have also isolated a bovine brain peptide with the same biological activity and the same molecular mass (1,810 ± 2) as the rat peptide. There was not enough of this peptide for complete sequencing, but 10 residues could be determined (4-8, 11, 12 and 14-16) that were identical to the corresponding amino acids in the rat sequence.

Several investigators have pointed out that the ORL_1 receptor might play a crucial role in pain perception [1, 4] and locomotion/curiosity control. Two in vivo observations now give experimental support to this notion :

(i) inhibition of receptor expression with an antisense oligonucleotide induces hypoalgesia (Fig. 1), and

(ii) intracerebroventricular administration of the heptadecapeptide induces hyperalgesia (Fig. 5), in mice.

Thus, the neuropeptide is endowed with pronociceptive properties and we suggest that it might be named nociceptin. Together, nociceptin and its receptor,

ORL_1, may represent the molecular basis to a novel pain regulatory modality in the central nervous system.

Finally, the striking structural homologies which exist between the ORL1 and opioid receptors and between the novel peptide and endorphins, make it likely that the genes encoding the two classes of receptors and those encoding the two classes of neuropeptides have evolved in parallel, each from a common ancestor. Since dynorphin A is one of several prodynorphin[16]-derived endorphins, one may anticipate that the novel peptide is but one representative of a larger family whose other members await identification.

As the ORL_1 is located in specific parts of the brain, such as the central nervous system, especially in limbic areas, hypothalamus, pons and spinal cord, said receptor and its ligand may be involved in the control of other functions and related behaviours. For instance, the ORL_1 receptor and its ligand may regulate neuroendocrine secretion, stress, learning and memory, attention and emotions, homeostasis and sensory perception, motricity (locomotion), anxiety, instinctive behaviour, curiosity, ...

Thus, the present invention is also related to any molecule which may affect said functions and behaviours and which is a ligand of the ORL_1 receptor.

2. Comparative organisation of the prepronociceptin (PPNOC) and opioid peptides precursor genes

The present invention is also related to the nucleic acid sequence having more than 70% homology with the nucleic acid sequence SEQ ID NO. 1 which encodes the peptide according to the invention.

Such a peptide is the prepronociceptin which may be cleaved into the two heptadecapeptides according to the

invention.

The figure 7 (upper panel) shows the general organisation of the prepronociceptin (PPMOC) genes. The PPMOC genes consist of 4 hexons (number I to IV) interspersed by 3 introns (A, B and C). The filled boxes correspond to the coding region. ATG, STOP and poly A are respectively the transcription start, stop and poly-adenylation sites.

The nucleotide sequence of the murine and human prepronociceptine (PPNOC) genes displays organizational and structural features which are very similar to those genes encoding the precursors to endogenous opioid peptides, enkephalins (PPENK), dynorphins/neo-endorphins (PPDYN) and ß-endorphin (PPOMC) (figure 7, lower panel). In particular, the translated region of the murine and human PPNOC genes is interrupted by an intron located between codons for amino acids 42 et 43. An intron is also present at the equivalent site in the PPENK, PPDYN and PPOMC genes. As it is the case in opioid peptide genes, another intron is also present in the 5' untranslated region of the PPNOC gene. In addition to these two introns shared with the other precursor genes, the PPNOC gene also includes an intron in the 3' untranslated region of the message, which is unusual. Nevertheless, the present data are compatible with the notion that the nociceptin and opioid peptides genes have evolved in parallel from a common ancestor.

The deduced amino acid sequence of prepronociceptin is highly conserved across murine and human species, especially the C-terminal quarter which hosts nociceptin itself. The N-terminal end of the precursor consists of a hydrophobic stretch of about 20 amino acids which may represent the signal peptide necessary for translocation into the rough endoplasmic reticulum, followed by a cystein-rich

portion which is also found in other hormone precursors, especially those to endogenous opioid peptides. Indeed, the pattern of cystein residues in pronociceptin is exactly the same as in proenkephalin and prodynorphin, suggesting a common mode of folding and/or processing of these precursor proteins. Lowest homology across murine and human prepronociceptins is observed in the core of the molecule with insertion of a variable number of repeated acidic motifs. The unique nociceptin sequence is located in the C-terminal fourth of the precursor where it is flanked by canonic Lys-Arg proteolytic excision motifs. Interestingly, pronociceptin contains other potential cleavage sites : one, Lys-Arg in the murine or Arg-Arg in the human sequence, is located upstream, and the other, Arg-Arg-Arg, downstream of nociceptin. Pronociceptin may therefore serve as the precursor not only to the two identified heptadecapeptides and the octapeptides but also to other physiologically important (neuro)peptides according to the invention.

3. Tissue distribution of rat prepronociceptin mRNAs

Northern blot analysis (figure 8) revealed that prepronociceptin messenger RNAs are present as a single species about 1.3 kb long in rat nerve tissue (brain and spinal cord) as well as in ovary. No signal could be detected in RNA extracts from peripheral tissues, including liver, intestine, stomach, lung, spleen, adrenal gland and testis.

4. Mapping of the human prepronociceptin gene

The human prepronociceptin gene was mapped by performing polymerase chain reactions on the radiation hybrid cell lines of the GeneBridge 4 panel. The Rhmapper program allowed to assigned unambiguously the gene to human

chromosome 8, between the STS markers WI-5833 and WI-1172 (figure 9).

5. Stimulation of locomotion and curiosity by nociceptin in mice

Early behavioral and functional studies indicated that the nociceptin peptide might exert awakening and psychostimulant actions in mice. The results confirm the preliminary observations by examining the effects of nociceptin on the horizontal and vertical components of locomotion as well as on exploratory behaviour. Since the ORL_1 receptor resembles opioids receptors and nociceptin dynorphin A, it has been verified that nociceptin-induced stimulation of locomotion in mice is insensitive to the opioate antagonist naloxone [22] and the locomotor effects of the peptide involve, as expected, dopamine transmission.

Material and methods

Animals

Male Swiss albino mice (CD1, Charles River, Saint Aubin lès Elbeuf, France) weighting 20-25 g were used in this study. They were housed 20 per box (L : 40 cm, W : 25 cm, H : 18 cm), with unlimited access to standard semi-synthetic laboratory food and tap water, under controlled environmental conditions (temperature : 22 ± 1 °C, 7 a.m. to 7 p.m. light-dark cycle). Experiments were carried out between 10 a.m. and 6 p.m. Each animal was used once.

Intracerebroventricular injections

Injections (10 µl) were performed free hand in the left ventricle [23]. The animals were routinely tested from the 5th minute after injection.

Locomotor activity

Locomotor activity was assessed using a Digiscan Animal Activity Monitor (Omnitek Electronics Inc.) This system consists of cages (L : 20 cm, W : 20 cm, H : 30 cm) surrounded by two surimposed sets each of 8 IR beam sensors, the lower set for monitoring horizontal and the upper, vertical deplacements, and interfaced with an Apple IIe computer. The cages were placed in dimly lit, sound-attenuated room.

Hole board test

The hole board test [24] consists in a plastic square plate (20x20 cm, 1 cm thick) with 16 holes, 2 cm in diameter, evenly spaced at 3.5 cm from the edges. The animals were placed in the centre of the plate and the number of head dips was measured during 4 consecutive periods of 5 minutes each.

Drugs and solutions

The nociceptin peptide according to the invention was solid phase synthesised. Naloxone hydrochloride was from Endo and SCH 23390 ® [25] from Schering. Haloperidol [26] solutions were obtained by dilution in saline (NaCl 0.9% w:v) of Haldol ® (Janssen).

Statistical analyses

The data are expressed as the mean ± standard error. Differences between groups were assessed by two-ways analysis of variance (ANOVA) and the Student's t-test. $P < 0.05$ was taken as the significant level of difference.

Results

The figure 10 shows that i.c.v. injection of a dose of nociceptin at low 10 ng/mouse elicited a statistically significant increase in horizontal locomotor activity. This effect was even more pronounced and, apparently, culminated at the dose of 100 ng. However, the stimulant action of the peptide lasted no more than 20 minutes. At higher doses (1 and 10 μg/mouse), nociceptin appeared to stimulate locomotion yet after a delay of at least 20 minutes following introduction into the monitor. The peptide also increased significantly verticalisation during the periods 0-10 and 10-20 minutes of observation at the doses of 100 ng and 1 μg. The results are expressed in the table 1.

Table 1

Nociceptin doses (ng/mouse)	Number of verticalisations during periods (min)		
	0-10 min	10-20 min	20-30 min
0	96 ± 24.3	131 ± 29.4	143 ± 33
10	195 ± 34	223 ± 39	222 ± 39
100	266 ± 41	253 ± 33.5	231 ± 34.5
1000	239 ± 56.2	301 ± 36.6	295 ± 50.5

In the hole board test, nociceptin (100 ng/mouse, i.c.v.) induced a significant increase in the number of explored holes (see table 2). During the 20 minutes period of observation, this number increased from 155 ± 15 in saline up to 226 ± 18 in peptide-treated animals($p<0.01$). On the contrary, when the animals were injected with morphin (5 mg/kg, s.c.) and tested 15 minutes later under the same conditions as nociceptin-treated animals, the number of explored holes was considerably reduced (150 ± 8 in saline vs 24 ± 2 in morphin-treated group ($p<0.001$)).

Naloxone (0.0-4.5 mg/kg, s.c.) injected 10 minutes before i.c.v. administration of 100 ng nociceptin did not suppress the motor-stimulant effect of the peptide observed during the first 10 minutes of testing (pretreatment x treatment interaction $F(1,20) = 0.023$) (figure 11).

In contrast, the D1 dopamine receptor antagonist SCH 23390 proved to be effective in antagonizing nociceptin stimulation of locomotor activity in mice (pretreatment x treatment interaction $F(1,20) = 4.25$ ($p<0.01$)) (figure 12). Similarly the D2 dopamine receptor antagonist haloperidol antagonized nociceptin-induces stimulation of locomotor activity (pretreatment x treatment interaction $F(1,20) = 2.57$; $p<0.05$) (figure 13).

Table 2

Treatments i.c.v.	Number of explored holes during the periods (min)			
	0-5	5-10	10-15	15-20
Saline	28.5±5.7	39.0±4.6	46.8±4.3	40.7±6.5
Nociceptin (100 ng/mouse)	50.4±6.8	64.5±7.3	62.4±5.6	58.6±6.6

The neuropeptide nociceptin stimulates both the horizontal and vertical components of the locomotion in mice. This effect is already seen at the low dose of 10 ng/mouse and seems to be effective at higher dose of 100 ng/mouse. Regardless of the dose, nociceptin's action appears to be of short duration, suggesting a rapid inactivation of the peptide under the experimental conditions. At the highest dose tested (10 μg), the stimulation of horizontal locomotor activity by nociceptin does not show up until at least 25 minutes following i.c.v. injection as it is exerted, during this period, an effect opposing those of low doses of the peptide. Since it has been previously emphasized that nociceptin resembles dynorphin A, which itself and other agonists of Kappa opioid receptors depress locomotion, one could argue that such opposing action at high dose of nociceptin might follow from the aspecific stimulation of these opioid receptors.

The stimulation of locomotor activity by nociceptin does not involve opioid receptors since the peptide displays none of the properties that are characteristic of morphin. In particular, the locomotor effect which is induced by nociceptin includes a vertical component and it does not correspond to the so-called "running fit" behaviour elicited

by the opiate. Neither does nociceptin induce the Straub tail phenomenon. Nociceptin is hyperalgesic in vivo and its stimulatory action on locomotion is not antagonized by the opiate antagonist naloxone.

Another large difference resides in the fact that nociceptin stimulates "curiosity" while morphin strongly decreases it. The motor-stimulant effect of nociceptin is unlikely to involve delta opioid receptor although delta opioid agonists are known to stimulate both the horizontal and vertical components of locomotion, yet this effect is reversed by naloxone and is accompanied by a naloxone-reversible analgesia in the hot plate test. Taken together, these data argue against the notion that the stimulant/ awakening actions of the nociceptin in mice involve opioid receptors.

Most psychostimulant agents operate through an increase in central dopaminergic transmission. To test this hypothesis, the interaction of nociceptin with the D2 dopamine receptor antagonist haloperidol and the D1 dopamine receptor antagonist SCH 23390 ® was considered. Antagonism was observes in each case, indicating that dopamine neurones, likely mesolimbic ones, are involved in nociceptin-elicited stimulation of locomotion.

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CLAIMS

1. Nucleic acid molecule which corresponds to at least 70% of the SEQ ID NO. 1 or its complementary strand.

2. Nucleic acid molecule which corresponds to at least 90% of the SEQ ID NO. 1 or its complementary strand.

3. Isolated nucleic acid molecule comprising at least the SEQ ID NO. 1, its complementary strand or a portion thereof, having more than 15 nucleotides able to identify or reconstitute SEQ ID NO. 1 or its complementary strain.

4. Peptide encoded by the nucleic acid molecule according to any of the preceding claims.

5. Peptide according to the claim 4, having the following amino acid sequence of SEQ ID NO. 2 : Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln, or agonists of its receptor(s).

6. Peptide according to the claim 5, characterized in that it is a ligand of the ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically the human ORL_1 receptor.

7. Peptide according to the claim 4, having the following amino acid sequence of SEQ ID NO. 3 : Phe-Ser-Glu-Phe-Met-Arg-Gln-Tyr-Leu-Val-Leu-Ser-Met-Gln-Ser-Ser-Gln, or agonists of its receptor(s).

8. Peptide according to the claim 4, having the following amino acid sequence of SEQ ID NO. 4 :

Thr-Leu-His-Gln-Asn-Gly-Asn-Val, or agonists of its receptor(s).

9. Inhibitor directed against the nucleic acid molecule according to any of the claims 1 to 4, the peptide according to any of the claims 4 to 8 or the receptor(s) of said peptide.

10. Inhibitor according to the claim 9, characterized in that it is a polyclonal or monoclonal antibody or a portion thereof, directed against the peptide according to any of the claims 4 to 8 or its receptor.

11. Inhibitor according to the claim 9, which is an antisense oligonucleotide which has a sequence capable of specifically binding to the nucleic acid molecule according to any of the claims 1 to 3 so as to prevent its transcription and/or its translation.

12. Inhibitor according to the claim 11, comprising chemical analogs of nucleotides.

13. Inhibitor according to the claim 11, said oligonucleotides having sequences which differ from one another at predefined positions.

14. Inhibitor according to any of the claims 11 to 13, wherein the oligonucleotide is coupled to a substance which inactivates the nucleic acid according to any of the claims 1 to 3.

15. Inhibitor according to the claim 14, wherein said substance is a ribozyme.

16. Inhibitor according to the claim 9, characterized in that it is an antagonist to the receptor of the peptide according to any of the claims 4 to 8.

17. Vector comprising the nucleic acid molecule according to any of the claims 1 to 3.

18. Pharmaceutical composition comprising an element chosen among the group consisting of the nucleic acid molecule according to any of the claims 1 to 3, the peptide according to any of the claims 4 to 8, the inhibitor according to any of the claims 9 to 16 and/or the vector according to the claim 17, and a pharmaceutically acceptable carrier.

19. Pharmaceutical composition comprising an amount of a substance effective to reduce the expression and/or the "effects" resulting from expression of the peptide according to any of the claims 4 to 8, and a pharmaceutically acceptable carrier.

20. Pharmaceutical composition comprising an amount of a substance effective to reduce the expression and/or the "effects" resulting from expression of the nucleic acid molecule according to any of the claims 1 to 3.

21. Pharmaceutical composition according to any of the claims 18 to 20, for the treatment and/or the prevention of a disease related to the following functions and/or behaviours : hyperalgesia, neuroendocrine secretion, stress, locomotor activity, anxiety, instinctive behaviour, decreasing of learning, memory, curiosity, attention and/or sensory perception.

22. Transgenic non-human animal which comprises the nucleic acid molecule according to any of the claims 1 to 3.

23. Method for recovering an inhibitor not known to be capable of specifically binding to a peptide according to any of the claims 4 to 8 can specifically bind to it, which comprises contacting the peptide according to any of the claims 4 to 8 under conditions permitting

binding of a inhibitor known to bind the peptide according to any of the claims 4 to 8, determining the presence of any inhibitor bound to said peptide and recovering said inhibitor.

24. Method for recovering a compound not known to be capable of specifically binding as an antagonist or as an agonist of the peptide according to the claim 6 to a ORL_1 receptor, preferably a mammal ORL_1 receptor, specifically a human ORL_1 receptor, can specifically bind to said receptor, which comprises contacting a cell, preferably a mammalian cell, comprising a vector adapted for expression in a mammalian cell, which vector further comprises nucleic acid molecule which expresses said ORL_1 receptor on the cell's surface, with the compound under conditions permitting binding of the peptide known to bind to said receptor, detecting the presence of any compound bound to said receptor, and recovering said compound.

25. Method for recovering a compound not known to be capable of specifically binding as an antagonist or as an agonist of the peptide according to the claim 6 to an ORL_1 receptor, preferably a mammal ORL_1 receptor, specifically a human ORL_1 receptor, can specifically bind to said receptor, which comprises preparing a cell extract from cells, preferably of mammalian cells, which comprises a vector adapted for expression in said cells, which vector further comprises nucleic acid molecule which expresses said receptor on the cell's surface, isolating a membrane fraction from the cells extract, incubating the compound with the membrane fraction under conditions permitting the binding of the peptide known to bind to said receptor, detecting the

presence of any bound compound, and recovering said compound.

26. Method for recovering a compound which is not known to be capable of binding as an antagonist or as an agonist of the peptide according to the claim 6 to an ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically a human ORL_1 receptor, and prevent the peptide according to the claim 6, to activate said receptor, which comprises contacting a cell, preferably a mammalian cell, which cell comprising a vector adapted for expression in said cell, such vector further comprising nucleic acid molecule which expresses said receptor on the cell's surface with the compound under conditions permitting measure of a functional response, determining whether the compound prevents the peptide to activate said receptor, and recovering said compound.

27. Method according to the claim 24, wherein the cell is a non-neuronal cell, comprising the cellular components necessary to produce a second messenger and wherein the determination (of whether the compound blocks the activation of the ORL_1 receptor by a peptide according to the claim 6 or mimics inactivation of the ORL_1 receptor by a peptide according to the claim 6) comprises detecting the change in the concentration of the second messenger.

28. Method according to the claim 27, wherein the second messenger is chosen among the group consisting of cyclic AMP (cAMP), inositol phosphate metabolite or intracellular calcium.

29. Method according to the claim 28, wherein the modification of the second messenger is monitored by a secondary production of a report molecule chosen among the group consisting of luciferase, -galactosidase,

chloramphenicol acetyltransferase or grove hormone, or by the physiological modification of the cell, preferably monitored by measure of the extra-cellular pH.

30. Method according to any of the claims 27 to 29, wherein the non-neuronal cell is CHO.

31. Compound identified by the method according to any of the claims 23 to 30.

32. Pharmaceutical composition comprising the compound according to the claim 31 and a pharmaceutically acceptable carrier.

33. Diagnostic and/or dosage device comprising an inhibitor according to any of the claims 9 to 16, the peptide according to any of the claims 4 to 8 and possibly its receptor(s), preferably the ORL_1 receptor.

34. Method of genetic treatment or prevention of a disease induced by the nucleic acid sequence or the peptide according to any of the claims 1 to 8 in an animal, specifically in a human, wherein an inhibitor according to any of the claims 9 to 16 or a nucleic acid molecule encoding said inhibitor is administered to a patient with a pharmaceutically acceptable carrier to reduce the expression and/or the "effects" resulting from expression of said nucleic acid sequence or said peptide.

chloramphenicol acetyltransferase or grove hormone, or by the physiological modification of the cell, preferably monitored by measure of the extra-cellular pH.

30. Method according to any of the claims 27 to 29, wherein the non-neuronal cell is CHO.

31. Compound identified by the method according to any of the claims 23 to 30.

32. Pharmaceutical composition comprising the compound according to the claim 31 and a pharmaceutically acceptable carrier.

33. Diagnostic and/or dosage device comprising an inhibitor according to any of the claims 9 to 16, the peptide according to any of the claims 4 to 8 and possibly its receptor(s), preferably the ORL_1 receptor.

34. Method of genetic treatment or prevention of a disease induced by the nucleic acid sequence or the peptide according to any of the claims 1 to 8 in an animal, specifically in a human, wherein an inhibitor according to any of the claims 9 to 16 or a nucleic acid molecule encoding said inhibitor is administered to a patient with a pharmaceutically acceptable carrier to reduce the expression and/or the "effects" resulting from expression of said nucleic acid sequence or said peptide.

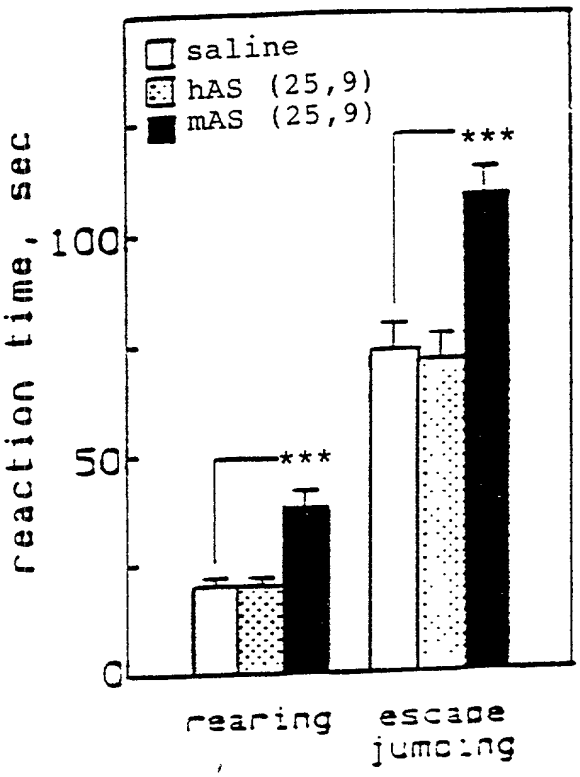
saline
hAS (25,9)
mAS (25,9)
reaction time, sec
100
50
0
***
***
rearing
escape jumping

FIG.1

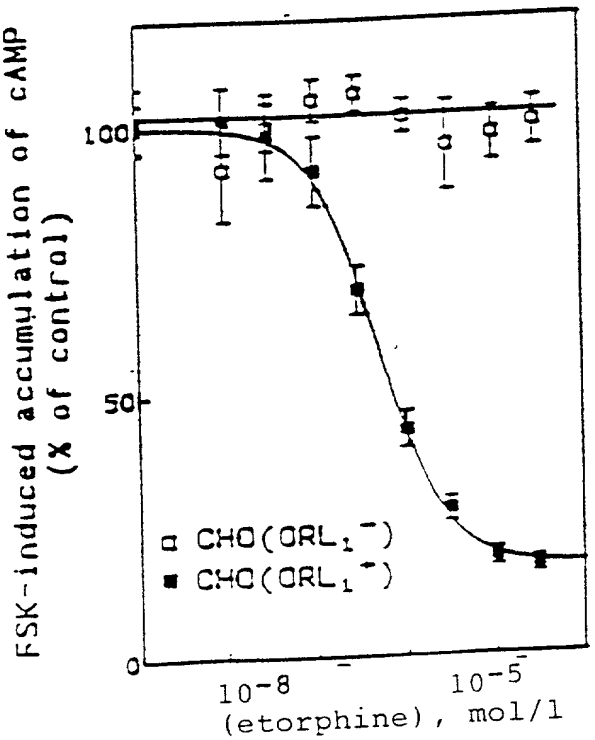
FSK-induced accumulation of cAMP (% of control)
100
50
0
CHO(ORL1+)
CHO(ORL1-)
10-8
10-5
(etorphine), mol/l

FIG.2

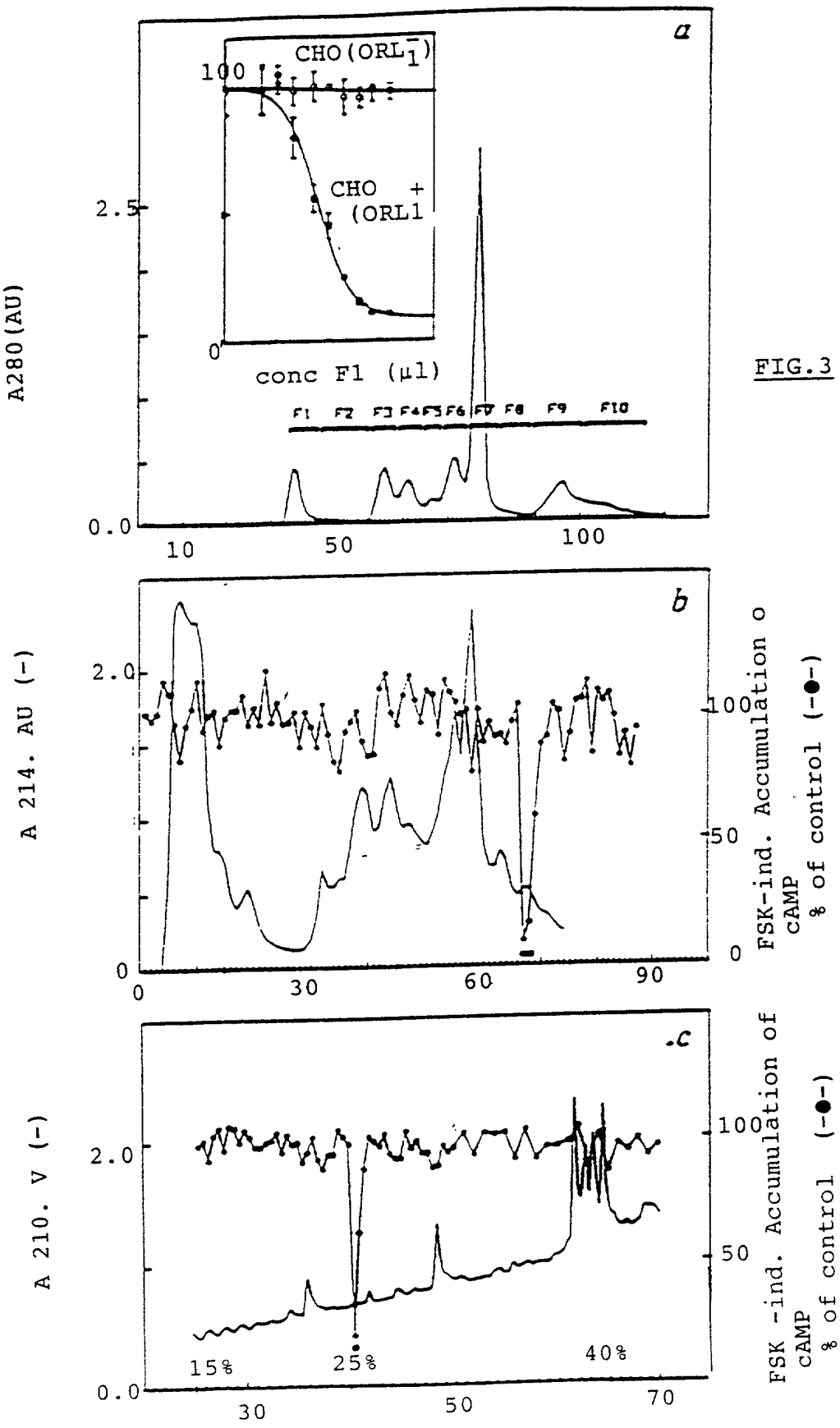
a
CHO(ORL1)
100
CHO + (ORL1
2.5
0
conc F1 (µl)
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10
A280(AU)
0.0
10
50
100
b
2.0
0
30
60
90
A 214. AU (-)
100
50
0
FSK-ind. Accumulation o cAMP
% of control (-●-)
c
2.0
0.0
15%
25%
40%
30
50
70
A 210. V (-)
100
50
FSK -ind. Accumulation of cAMP
% of control (-●-)

FIG.3

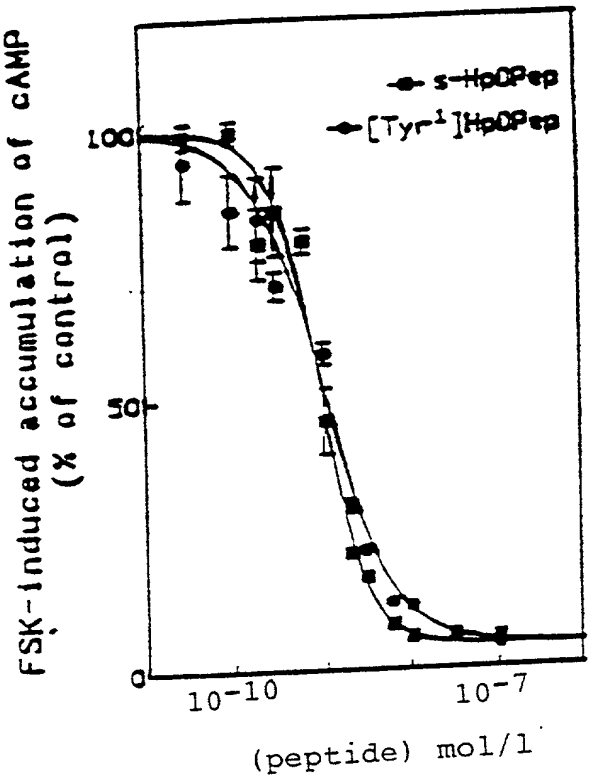

FIG.4

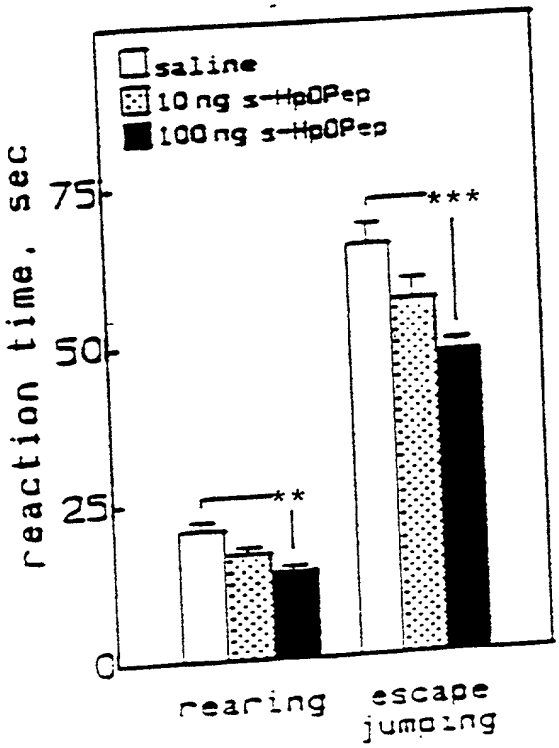

FIG.5

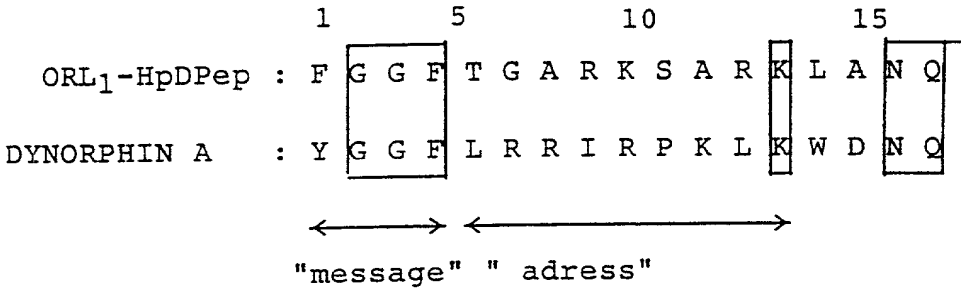

FIG.6

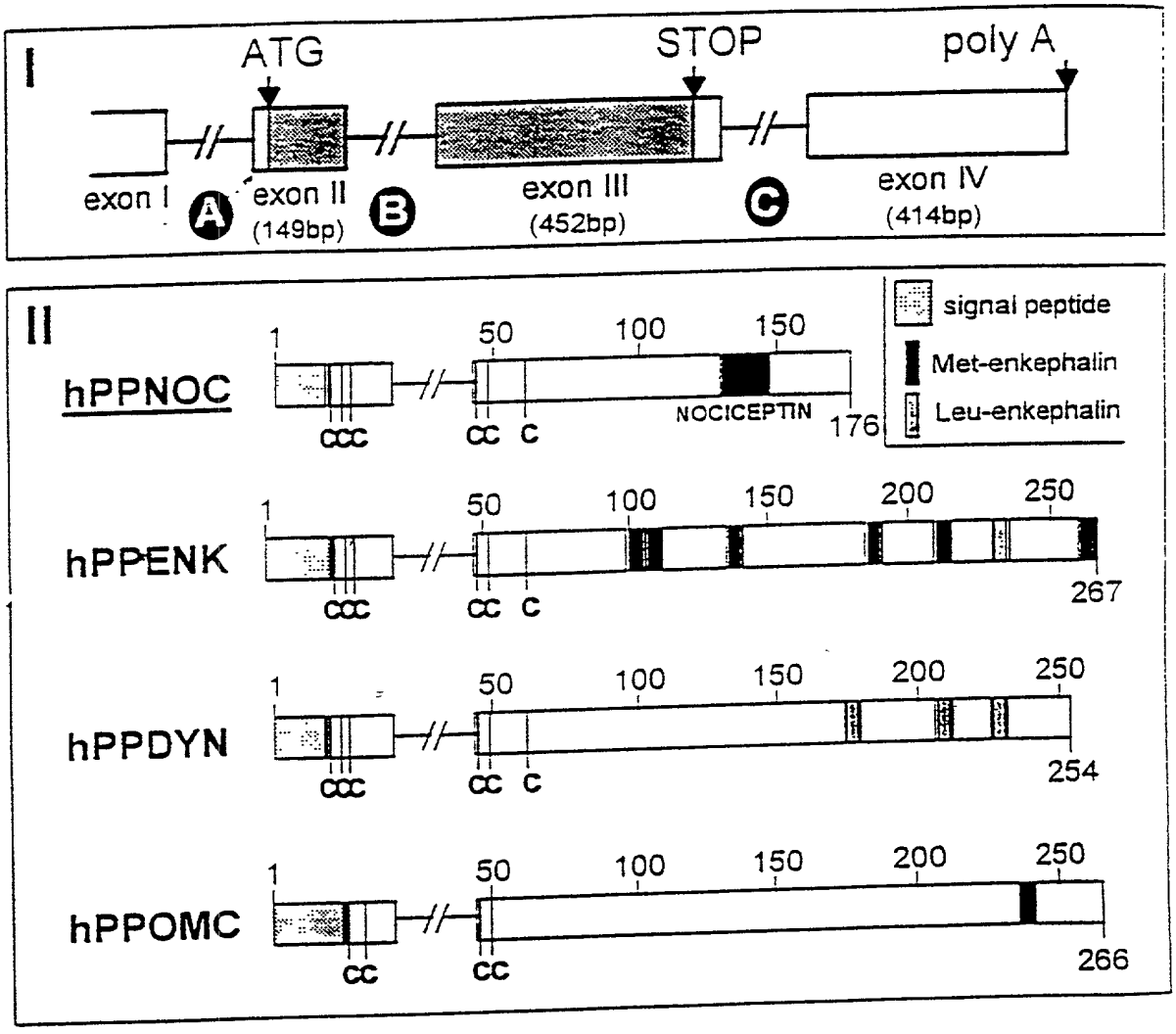
I
ATG
STOP
poly A
exon I
exon II
(149bp)
exon III
(452bp)
exon IV
(414bp)
A
B
C
II
signal peptide
Met-enkephalin
Leu-enkephalin
hPPNOC
NOCICEPTIN
176
hPPENK
267
hPPDYN
254
hPPOMC
266
1
50
100
150
200
250

FIG. 7

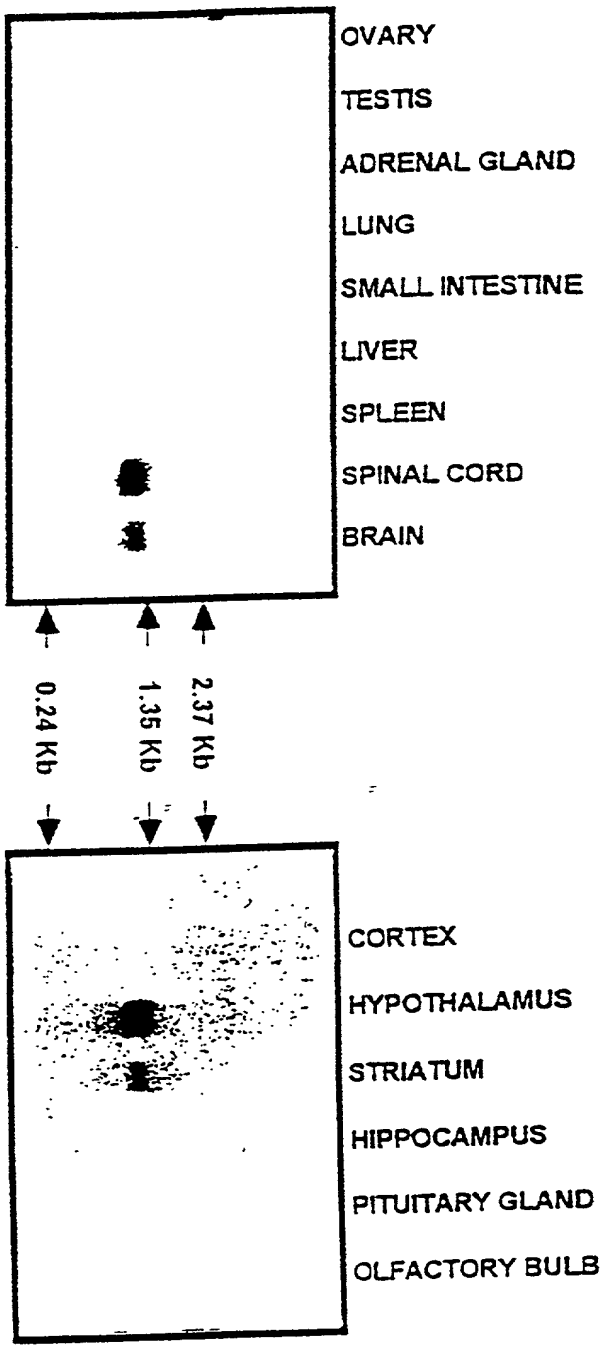

FIG. 8

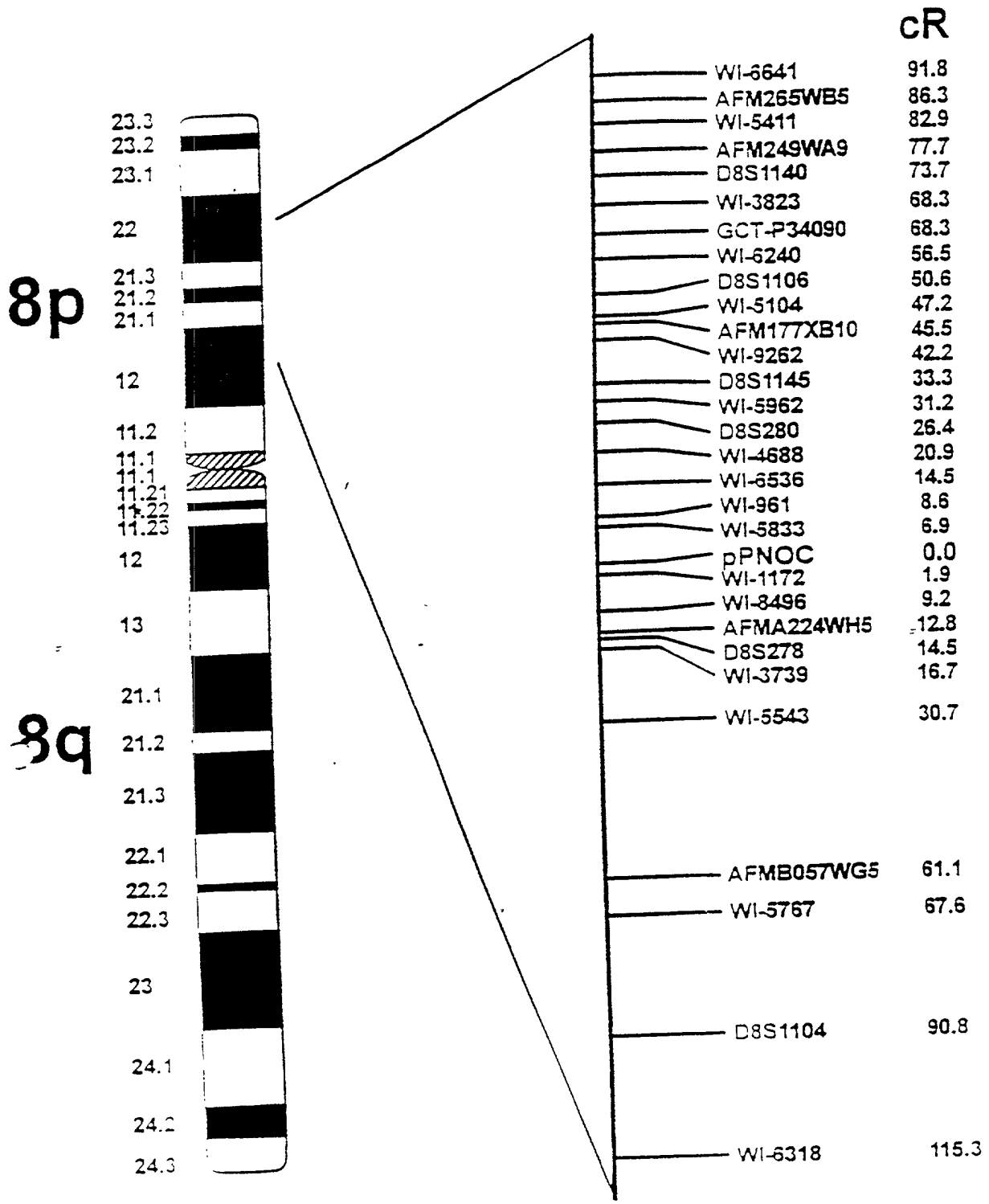

FIG. 9

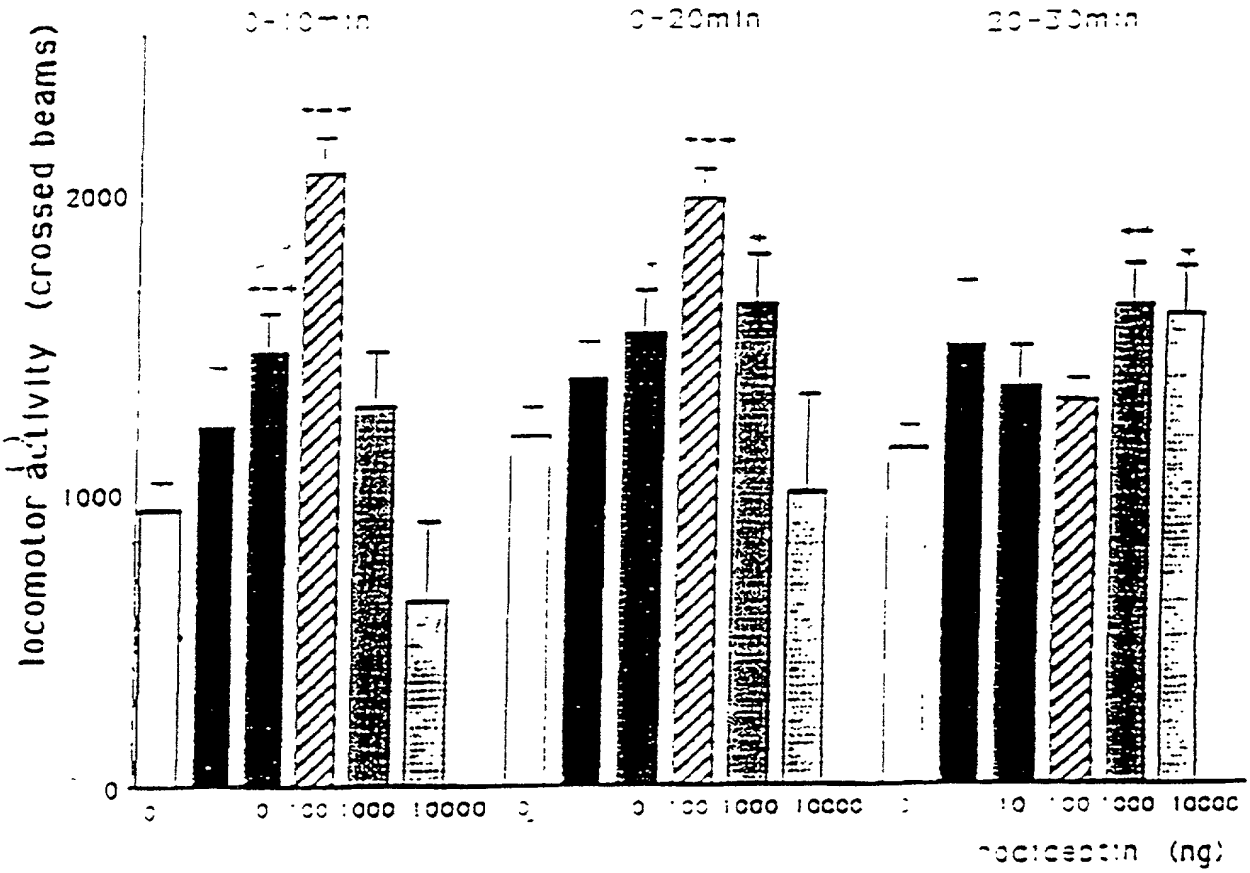
0-10min
0-20min
20-30min
locomotor activity (crossed beams)
2000
1000
0
0
100
1000
10000
nociceptin (ng)

FIG. 10

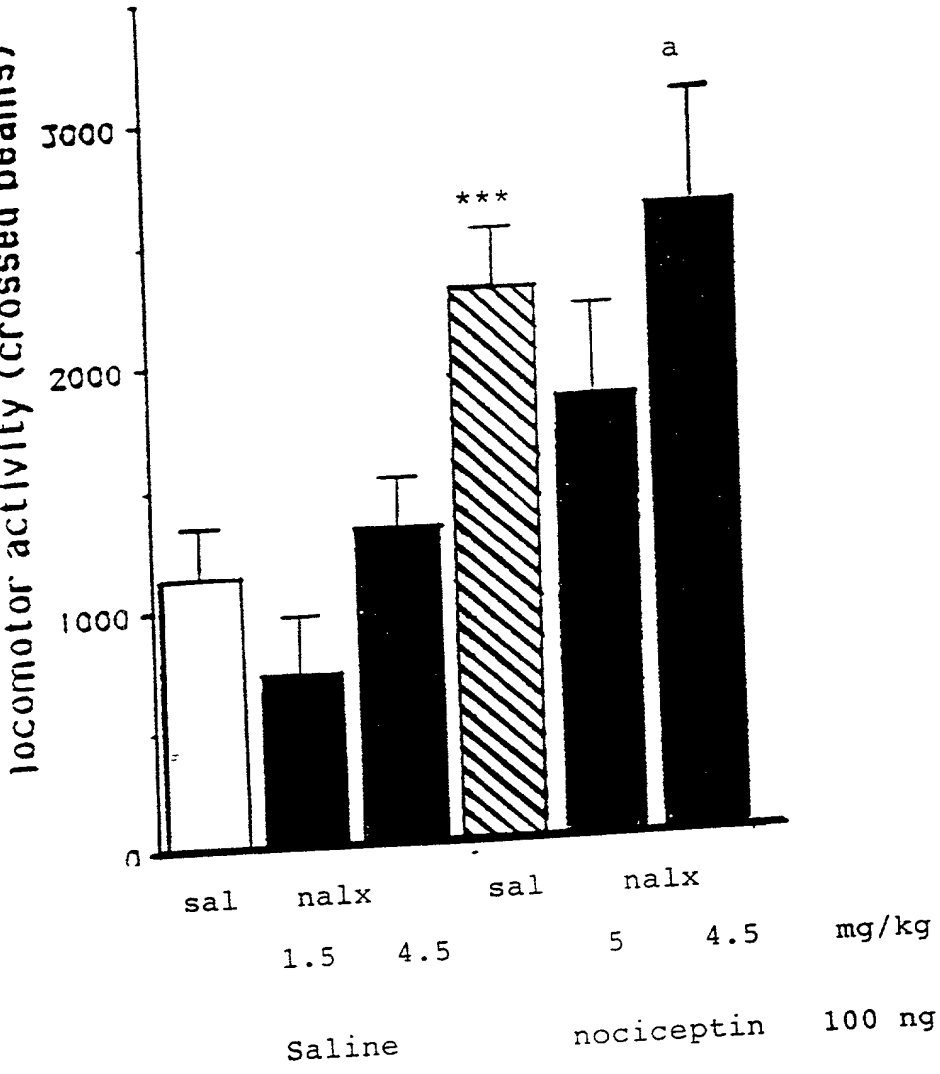

FIG.11

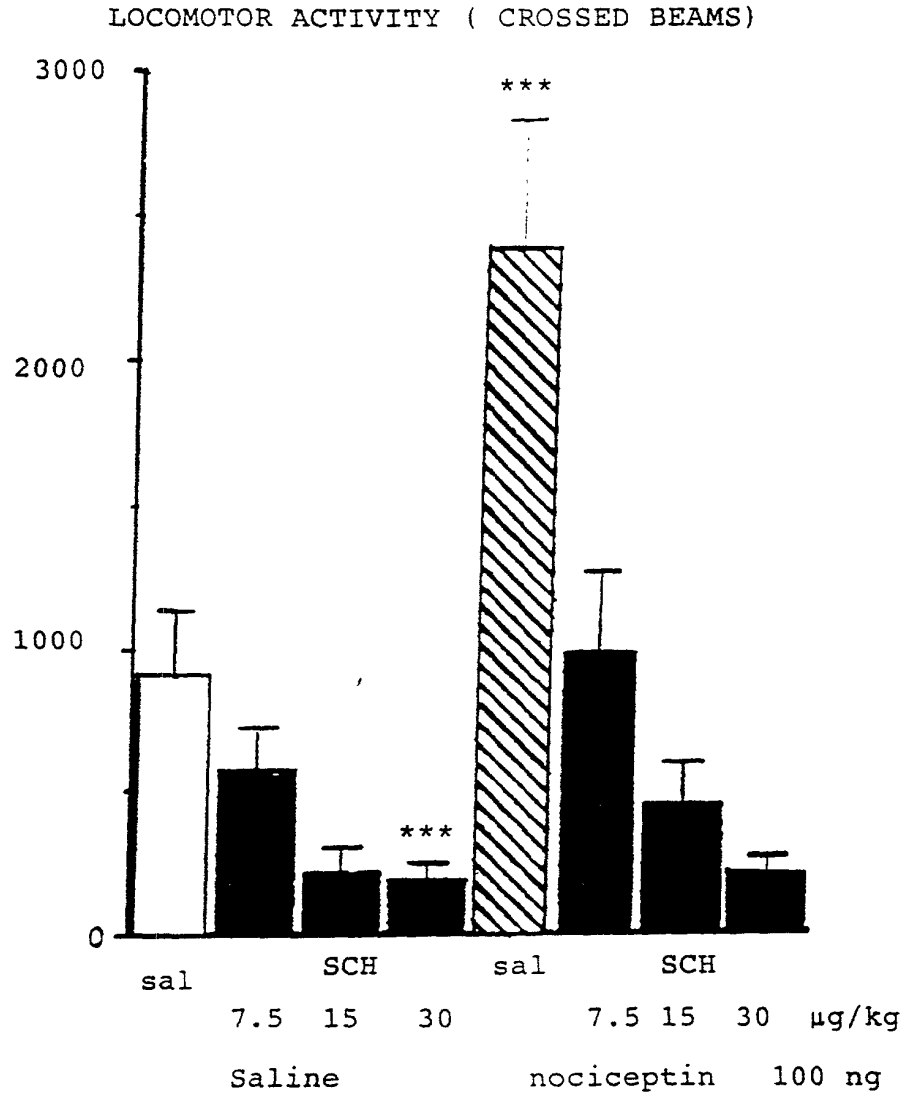
LOCOMOTOR ACTIVITY (CROSSED BEAMS)
3000
2000
1000
0
***
***
sal
SCH
sal
SCH
7.5 15 30
7.5 15 30 µg/kg
Saline
nociceptin 100 ng

FIG.12

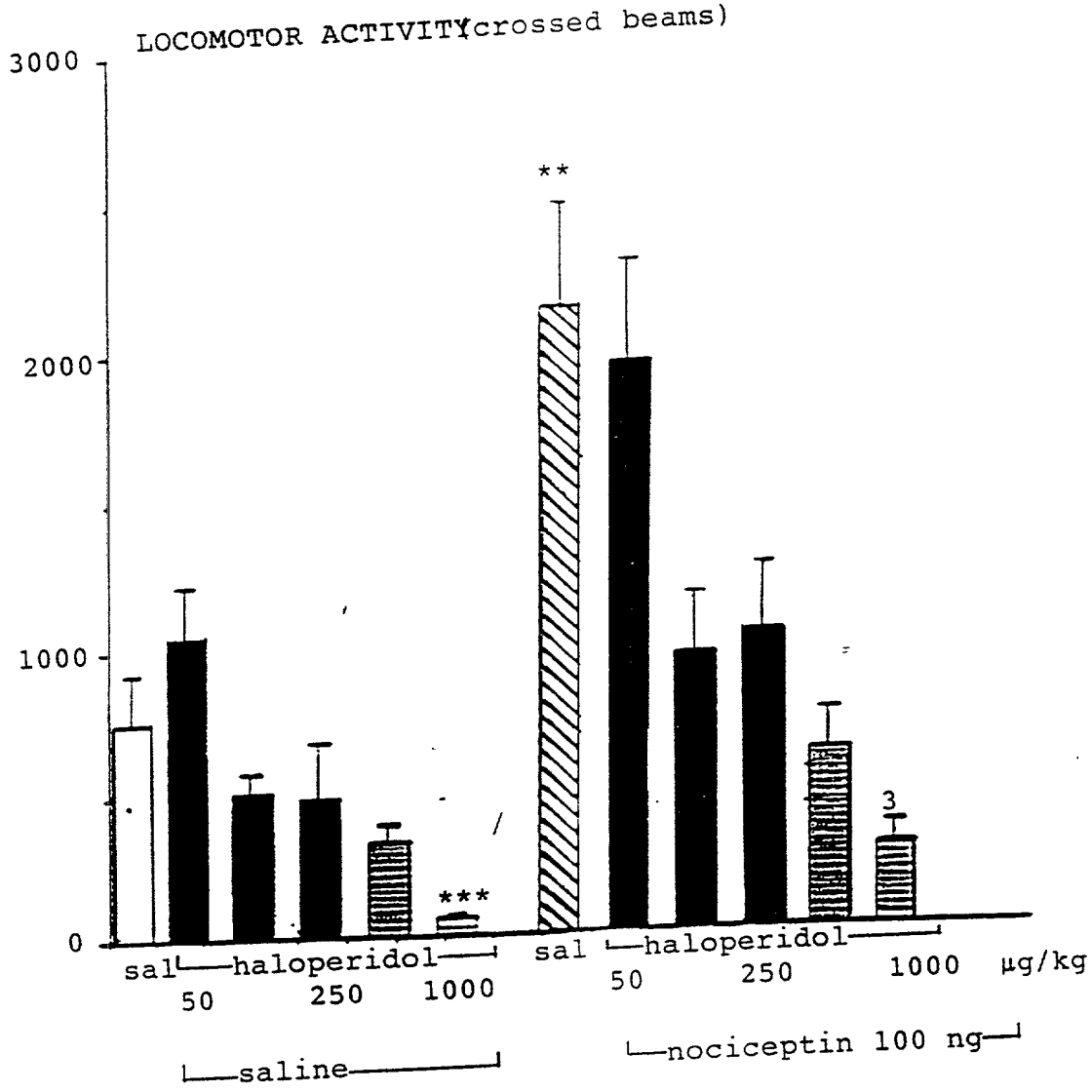
LOCOMOTOR ACTIVITY (crossed beams)
3000
2000
1000
0
**
***
3
sal
haloperidol
50
250
1000
sal
haloperidol
50
250
1000
µg/kg
saline
nociceptin 100 ng

FIG. 13

P.ULB.33/US(ab)

Declaration and Power of Attorney for Patent Application

Déclaration et Pouvoirs pour demandes de brevet

French Language Declaration

En tant que l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité figurant ci-dessous à côté de mon nom,

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) du sujet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée :

et dont les caractéristiques sont fournies ci-joint à moins que la case suivante n'ait été cochée :

O a été déposé le
sous le numéro de Demande des Etats-Unis ou
sous le numéro de demande internationale PCT et modifiée le
(le cas échéant).

Je déclare par le présent acte avoir passé en revue et pris connaissance du contenu des caractéristiques ci-dessus, revendications comprises, telles que modifiées par tout amendement dont il aura été fait référence ci-dessus.

Je reconnais de voir divulguer toute information pertinente à l'examen de cette demande, comme le définit le Titre 37, §1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled :

Nucleic acid molecules encoding peptides having pronociceptive properties.

the specification of which is attached hereto unless the following box is checked :

O was filed on
as United States Application Number or PCT International Application Number
and was amended on
(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentibility as defined in Title 37, Code of Federal Regulations, § 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119 du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur figurant ci-dessous et ai aussi pris connaissance de toute demande étrangère de brevet ou de tout certificat d'inventeur ayant une date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior foreign applications
Demande(s) de brevet antérieure(s)

Priority claimed
Droit de priorité revendiqué

			O	O
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	Yes Oui	No Non
60/002 368	***U.S.A.***	***15/08/1995***	●	O
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	Yes Oui	No Non
			O	O
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	Yes Oui	No Non

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis figurant ci-dessous et, dans la mesure où le sujet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande américaine préalable, en vertu des dispositions de premier paragraphe du Titre 35, § 112 du Code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la demande de brevet comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la première demande et la date de dépôt de la demande nationale ou PCT internationale :

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentibility as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application :

(Application Serial No.) (No. de série de la demande)	(Filing date) (Date de dépôt)	(Statut) (Breveté, en attente, annulé)	(Status) (Patented, pending, abandoned)
(Application Serial No.) (No. de série de la demande)	(Filing date) (Date de dépôt)	(Statut) (Breveté, en attente, annulé)	(Status) (Patented, pending, abandoned)

Je déclare par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la Section 1001 du Titre 18 du Code des Etats-Unis et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocat(s) et/ou agent(s) suivant(s) pour qu'il(s) poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire avec le Bureau des brevets et marques s'y rapportant.
(mentionner le nom et le numéro d'enregistrement)

POWER OF ATTORNEY : As named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and trademark Office connected there with.

(list name and registration number)

Adresser toute correspondance à :

Send Correspondence to :

Adresser tout appel téléphonique à :
(nom et numéro de téléphone)

Direct Telephone Calls to :
(name and telephone number)

Nom complet de l'unique ou premier inventeur	Full name of sole or first inventor ***PARMENTIER Marc***
Signature de l'inventeur Date	Inventor's signature Date 26/06/1998
Domicile	Residence ***Chaussée d'Uccle 304*** ***B-1630 LINKEBEEK*** ***BELGIUM***
Nationalité ***Belgian***	Citizenship ***Belgian***
Adresse postale	Post Office Address ***Chaussée d'Uccle 304*** ***B-1630 LINKEBEEK*** ***BELGIUM***

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire)

(Supply similar information and signature for any subsequent joint inventor)

Nom complet du second co-inventeur, le cas échéant	Full name of second joint inventor, if any
2	***VASSART Gilbert***
Signature du second inventeur Date	Second inventor's signature Date 26/06/1998
Domicile	Residence ***Avenue Lambeau 113*** ***B-1200 BRUSSELS*** ***BELGIUM*** BEX
Nationalité	Citizenship *Belgian*
Adresse postale	Post Office Address ***Avenue Lambeau 113*** ***B-1200 BRUSSELS*** ***BELGIUM***

Nom complet du troisième co-inventeur, le cas échéant	Full name of third joint inventor, if any
3	***MEUNIER Jean-Claude***
Signature du second inventeur Date	Third inventor's signature Date 26/06/1998
Domicile	Residence ***Lotissement du pin 8*** ***F-31320 REBIGUE*** FRX ***FRANCE***
Nationalité	Citizenship *French*
Adresse postale	Post Office Address ***Lotissement du pin 8*** ***F-31320 REBIGUE*** ***FRANCE***

Nom complet du troisième co-inventeur, le cas échéant	Full name of third joint inventor, if any
	MOLLEREAU Catherine
Signature du second inventeur Date	Third inventor's signature Date 26/06/1998
Domicile	Residence ***Chemin des Saules 15*** ***F-31320 CASTANET*** FRX ***FRANCE***
Nationalité	Citizenship *French*
Adresse postale	Post Office Address ***Chemin des Saules 15*** ***F-31320 CASTANET*** ***FRANCE***